RESEARCH ARTICLE

Do colonization by dark septate endophytes and elevated temperature affect pathogenicity of oomycetes?

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Abstract

Phialocephala subalpina is one of the most frequent dark septate root endophytes in tree roots but its function in forest ecosystems is largely unknown. A full-factorial infection experiment was performed, using six *P. subalpina* isolates, two pathogenic oomycetes (*Phytophthora plurivora* [syn. *Phytophthora citricola* s.l.] and *Elongisporangium undulatum* [syn. *Pythium undulatum*]) and two temperature regimes (17.9 and 21.6 °C) to examine the ability of *P. subalpina* to protect Norway spruce seedlings against root pathogens. Seedling survival, disease intensity and seedling growth were affected by *P. subalpina* genotype, temperature and pathogen species. Some *P. subalpina* isolates effectively reduced mortality and disease intensity caused by the two pathogens. Elevated temperature adversely affected seedling growth but did not aggravate the effect of the pathogens. *Elongisporangium undulatum* but not *P. plurivora* significantly reduced plant growth. Colonization density of *P. subalpina* measured by quantitative PCR was not affected by temperature or the presence of the pathogens. In conclusion, *P. subalpina* confers an indirect benefit to its host and might therefore be tolerated in natural ecosystems, despite negative effects on plant health and plant growth.

Introduction

Microorganisms are interacting in complex ways with plants and each other in natural communities. Aerial plant surfaces are colonized by epiphytic microorganisms, the rhizosphere hosts a plethora of soil microorganisms, and most functional plant tissues are colonized by endosymbionts. Microbial endosymbionts are ubiquitous, and depending on their effects on the host, they are considered as mutualistic, pathogenic or neutral. However, the nature of host–endosymbiont interactions is usually not fixed and depends on a multitude of factors, such as environmental conditions, time of observation of the interaction or host and microorganism genotype. For instance, mycorrhizal fungi reduce plant growth during the early establishment of the symbiosis compared to mycorrhiza-free plants, but when the mycorrhiza is functional, these plants gain relatively more biomass and overgrow nonmycorrhized plants (Johnson *et al.*, 1997). In contrast, the nature of host–endosymbiont interactions is less clear for nonmycorrhizal endosymbionts.

Fungal endophytes are endosymbionts that colonize most functional plant tissues but do not cause disease symptoms, either for a prolonged period of time or never (Saikkonen *et al.*, 1998; Brundrett, 2004). Some endophytes can have beneficial effects on their plant hosts, such as increased tolerance to drought, heat or high levels of metal concentrations (Clay, 2001; Rodriguez *et al.*, 2009), the production of compounds that are toxic for herbivores or make the infected tissues unpalatable for them or the deterrence of harmful pathogens (Carroll, 1988; Clay, 2001; Arnold *et al.*, 2003; Selosse *et al.*, 2004; Sieber, 2007; Miller *et al.*, 2008). Whilst these effects have been relatively well studied for common leaf and needle endophytes, the ecological significance of naturally occurring root endophytes in protecting plants against pathogens is not well understood.

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Yet, the presence of fungal root endophytes in plants, in particular of dark septate endophytes (DSE), is very common. DSE are abundant root colonizers of a wide range of mycorrhizal and nonmycorrhizal plant species (Stoyke *et al.*, 1992; Sieber, 2002; Addy *et al.*, 2005) and form a polyphyletic group of ascomycete fungi with melanized, septate hyphae (Stoyke *et al.*, 1992; Ahlich & Sieber, 1996; Jumpponen & Trappe, 1998; Newsham, 2011). In conifers and ericaceous shrubs, the most prevalent DSE fungi belong to the *Phialocephala fortinii* s.l.–*Acephala applanata* cryptic species complex (PAC) (Wang & Wilcox, 1985; Ahlich & Sieber, 1996; Grünig *et al.*, 2008). All known PAC species are widely distributed across the northern hemisphere without showing any biogeographic pattern (Queloz *et al.*, 2011). PAC communities are composed of up to ten species, but species composition neither correlates with host species (Ahlich & Sieber, 1996; Addy *et al.*, 2005; Grünig *et al.*, 2008; Walker *et al.*, 2011) nor with climate and is assumed to be mainly driven by stochastic effects (Queloz *et al.*, 2011). Despite the wide distribution and frequent occurrence, PAC behaves in a range from nearly neutral to highly virulent on Norway spruce seedlings, showing only small differences among species (Grünig *et al.*, 2008; Tellenbach *et al.*, 2011). One possible explanation for this apparent contradiction might be that PAC provides indirect benefits to their host by protecting it against harmful root pathogens. Such protection could be vital for plant seedlings, as these are particularly susceptible to pathogens (Newhook & Podger, 1972). In nurseries, damping-off of conifer seedlings occurs frequently and is often due to oomycete root pathogens of the genera *Pythium* and *Phytophthora* (Hendrix & Campbell, 1973; Hamm & Hansen, 1982; Lilja *et al.*, 1992). Moreover, oomycetes are globally involved in dieback of forest trees (Newhook & Podger, 1972; Brasier, 1996; Jung *et al.*, 1996, 2005; Nechwatal & Osswald, 2001; Brasier *et al.*, 2004; Chavarriaga *et al.*, 2007). Two widely distributed species are *Elongisporangium undulatum* (syn. *Pythium undulatum*, Uzuhashi *et al.*, 2010) and *Phytophthora plurivora* (syn. *Phytophthora citricola*, Jung & Burgess, 2009). *Elongisporangium undulatum* is often found in nurseries causing remarkable loss to pine and spruce seedlings (Lilja *et al.*, 1992), and it was shown to cause disease and mortality in conifer seedlings in infection trials (Lilja, 1994; Shafizadeh & Kavanagh, 2005). *Phytophthora plurivora* is primarily known as a pathogen of broadleaved tree species (Jung *et al.*, 1996, 2005; Nechwatal & Osswald, 2001; Jung & Burgess, 2009), but can also cause significant root loss and mortality in Norway spruce seedlings (Nechwatal & Osswald, 2001). Oomycetes seem to react strongly to environmental conditions (Newhook & Podger, 1972), and might gain even more importance under climate change, as warming combined with high amounts of rainfall is predicted to create favourable conditions for their growth and development (Brasier, 1996; Desprez-Loustau *et al.*, 2007). The effects of climate change on different pathosystems are variable, but diseases in general are expected to become more damaging (Ayres & Lombardero, 2000; Harvell *et al.*, 2002; Garrett *et al.*, 2006; Walther, 2010). The effect of climate change on neutral or beneficial interactions is little understood, but is also predicted to vary considerably (Compant *et al.*, 2010; Van der Putten *et al.*, 2010).

Norway spruce forests are considered to be highly vulnerable to climate change (Ohlemüller *et al.*, 2006), and the question arises whether disturbance of the endophyte –pathogen–host equilibrium might enhance this effect. Thus, the goal of this study was to examine in a tripartite host–endophyte–pathogen system whether *Phialocephala subalpina* can protect Norway spruce seedlings against *P. plurivora* and *E. undulatum* and whether this effect is altered by elevated temperature.

Materials and methods

Host plant and fungal isolates

The experiment was performed with Norway spruce (*Picea abies*) seedlings from a central alpine provenance (Fully, Switzerland) and six genetically distinct PAC isolates representing different multilocus microsatellite haplotypes (6_16_1, 6_2_7v, 6_37_6v, 6_53_6v, 6_70_4, 6_8_7v) (Queloz *et al.*, 2010). These isolates belong to the globally most widely distributed PAC species, *P. subalpina* (Queloz *et al.*, 2011), and originate from a single population (Bödmeren, Switzerland). Moreover, they have previously been shown to vary in strength of interaction with Norway spruce from neutral to highly virulent (Tellenbach *et al.*, 2011). *Phytophthora plurivora* (syn. *P. citricola*) isolate Bu 137/7a (Nechwatal & Osswald, 2001; Jung & Burgess, 2009) and *E. undulatum* (syn. *P. undulatum*) (CBS 101728) (Uzuhashi *et al.*, 2010) were included as soilborne root pathogens.

Experimental procedures

Phialocephala subalpina isolates were grown on terramycine-malt agar [TMA; 15 g L^{-1} malt extract (Hefe Schweiz AG, Stettfurt, Switzerland), 20 g L^{-1} agar, 50 mg L^{-1} terramycine] in Petri dishes at 20 °C in the dark. After 1 week, one colonized agar plug (diameter = 4 mm) from the margin of the growing colony was transferred to 50 mL 2% malt broth (20 g L^{-1} malt extract) in 100-mL Erlenmeyer flasks and incubated at 20 °C on a rotary shaker at 100 r.p.m. After 23 days, the

mycelium was harvested and washed with sterile nanopure water. Mycelia were blotted dry on a sieve and weighed. Then, they were homogenized with a blender for 30 s, and the thallus-to-water ratio was adjusted to 55 g L^{-1} (fresh weight) with sterile nanopure water. Fifty millilitre Falcon tubes containing a sterile 1 : 1 : 1 (v/v/v) silica sand/vermiculite/sphagnum peat mixture and a 1-mL pipette tip as spacer for the addition of *P. plurivora* or *E. undulatum* later on were inoculated with 1 mL of the homogenized *P. subalpina* mycelium and rinsed with 3 mL sterile nanopure water to distribute the inoculum more evenly in the substrate, whereas 4 mL sterile nanopure water was added to uninoculated control tubes. Then, tubes were incubated for 14 days at 20 °C in the dark. Thirteen-day-old sterile spruce seedlings were planted in the tubes. Seedlings had been produced from surface-sterilized seeds to exclude seedborne fungi and bacteria. Surface sterilization occurred by immersion in 30% H_2O_2 for 30 min, followed by rinsing with sterile nanopure water. Germination occurred within 12 days on water agar at 18 °C in the dark. After planting the seedlings into the Falcon tubes, plants were transferred to a phytotron [16-h day (120–140 μE m^{-2} s^{-1})/8-h night, temperature (24 °C/15 °C) and relative humidity (rH 45%/rH 85%)]. Tubes were randomly distributed in non-heated or heated water baths to expose them to two different temperature regimes. Thus, temperature in the tubes fluctuated between 21.0 and 14.7 °C (day/night; mean 17.9 °C) for the low temperature treatment, corresponding to average June temperatures recorded at the climate-measuring station in Sion (http://www.meteoschweiz.admin.ch/), which lies about 20 km apart from the seed origin in Fully, and between 26.2 and 16.5 °C (day/night; mean 21.6 °C) for the elevated temperature regime. After planting, all seedlings were fertilized with 5 mL of a 0.2% dilution (v/v) of complete fertilizer (Wuxal, Maag, Switzerland). Thereafter, plants exposed to low temperature were watered every other day with 3–4 mL deionized water. Plants exposed to elevated temperature were watered equally but once a week they were given an additional 1 mL deionized water to compensate for higher evaporation. In the first month, plants of both treatments were given 4 mL 0.2% complete fertilizer weekly and thereafter once every 3 weeks.

The inoculum of *P. plurivora* and *E. undulatum* was prepared as follows. Two colonized plugs were punched out with a cork borer (diameter = 4 mm) from the margin of 3-day-old cultures growing on 10% carrot juice agar (CA; 100 mL L^{-1} carrot juice, 20 g L^{-1} agar, pH = 7.0) plates and used to inoculate sterile vermiculite–millet–carrot juice [VMC, vermiculite/millet/carrot juice = 50 : 4 : 35 (v/v/v)] inoculation medium in 50-mL Falcon tubes. The cultures were incubated at room temperature. After 2 months, the VMC medium was rinsed with nanopure water to remove excess nutrients and used for inoculation. Inoculation of the seedlings with *P. plurivora* and *E. undulatum* occurred 49 days after planting. The pipette tip was removed and replaced by 1 mL inoculated VMC medium, and controls received sterile VMC medium. Then, tubes were flooded with deionized water for 72 h to induce sporangia formation of *P. plurivora* and *E. undulatum*. Thereafter, watering and fertilizing occurred as described above. We applied a full-factorial experimental design, consisting of two different temperature regimes with six different *P. subalpina* isolates and an uninoculated control treatment and with two pathogens and a sterile inoculate. Each treatment was replicated 10 times, resulting in 420 experimental units.

Data collection

Twenty-two days after pathogen inoculation, virulence, that is, the degree of damage caused to the seedlings, was assessed as disease intensity and reduction of seedling performance. Disease intensity, which includes the two components disease incidence, that is, the number of affected seedlings (e.g. mortality rate), and disease severity, that is, the percentage of necrotic or chlorotic surface area of the needles, was scored: (0) seedlings without any apparent disease symptoms, (1) seedlings with < 50% of the needle surface necrotic, (2) seedlings with more than 50% of the needle surface necrotic or chlorotic, (3) seedlings dead. Dead seedlings were removed and processed as described below. Scoring of the seedlings and removal of dead plants was repeated weekly. The experiment was terminated 70 days after pathogen inoculation, disease intensity was scored again, and plants were harvested.

The shoot was cut off, and roots were washed under running tap water and scanned on a standard flatbed scanner to measure root lengths, using the root analysis software WinRhizo (Pro 2009c, Regent Instrument Inc., Canada). Then, three 5-mm-long root segments were excised from the root system of all dead seedlings that had been removed during the experiment and from two randomly chosen seedlings at harvest: one from the periphery of the root system, one from the middle of the root system and one close to the hypocotyl. Two segments were surface-disinfected (1 min in 30% H_2O_2, 10 s in 98% EtOH) and incubated on TMA to re-isolate *P. subalpina* and the third one without surface disinfection was laid on PARP medium (Jeffers & Martin, 1986) to re-isolate *P. plurivora* and *E. undulatum* to verify Koch's postulates. Similarly, from the same two seedlings and an additional seedling per treatment, four 5-mm-long root segments from the same root regions (an extra segment was excised from the middle of the root system)

were pooled, freeze-dried, weighed and analysed, using a nested quantitative PCR (qPCR) to estimate the colonization density of *P. subalpina* (Tellenbach *et al.*, 2010). Afterwards, the seedlings were dried in an oven at 50 °C for two consecutive days, and the following plant growth–related parameters were measured: total biomass, needle mass, shoot mass, root mass, the root-to-shoot ratio (R/S ratio, i.e. root mass divided by shoot mass) and specific root length (SRL, i.e. root length divided by root mass).

DNA extraction and qPCR conditions

Conditions for DNA extraction and qPCR were the same as in the study by Tellenbach *et al.* (2010): samples were frozen at −80 °C and freeze-dried for 2 days. A few grains of DNA-free silica sand (dried for 4 h at 180 °C) were added to the lyophilized root samples to facilitate the disruption of plant cell walls. Then, samples were frozen again at −80 °C and homogenized using a bead mill. DNA was extracted using a CTAB/DNeasy plant mini kit (Qiagen, Basel, Switzerland) extraction, following the protocol of Grünig (2003), with adjustments for small sample sizes: only 250 μL lysis buffer and 3 μL RNAse A were added to the ground tissue, and there was only one washing step with 500 μL chloroform–isoamyl alcohol (1 : 24).

A nested qPCR was performed using the external primers pPF-076_F1 and pPF-076_R1 (Grünig *et al.*, 2007) in step 1 and the qPCR primers and probe pPF-076_qPCR_F, pPF-076_R, pPF-076_P (Tellenbach *et al.*, 2010) in step 2. The PCR in step 1 was performed in a total volume of 25 μL containing 5 μL 1 : 25 diluted sample DNA, 2.5 μL 10× PCR buffer (GE, Switzerland), 200 μM dNTPs, 0.5 units of Taq polymerase (GE, Switzerland) and 500 nM each external primer. PCR amplification conditions were as follows: one cycle at 94 °C for 2 min; 15 cycles at 94 °C for 30 s, 60 °C for 45 s and 72 °C for 30 s; and a final cycle at 72 °C for 6 min. In step 2, reactions were performed in a total volume of 25 μL containing 5 μL of the PCR product of step 1, 12.5 μL of 2× reaction buffer (qPCR MasterMix Plus Low ROX; Eurogentec, Belgium), 600 nM each primer and 150 nM probe. PCR amplification conditions consisted of a denaturation step at 95 °C for 10 min, 40 cycles at 95 °C for 15 s and 60 °C for 1 min, fluorescence was monitored during the 60 °C phase, as a standard serially diluted genomic DNA with known concentrations was added to each sample plate, and each sample was run in triplicate.

Statistical analyses

All statistical analyses were performed using the R statistical package (R Development Core Team, 2009). Seedling survival in relationship to temperature, *P. subalpina* isolate and pathogens was analysed in a survival analysis, with a Cox proportional hazard model using the *coxph* function in the *Survival* library. An individual plant was censored in the analysis when it survived until the end of the experiment. Similarly, ordinal categories of disease intensity were analysed with a proportional odds model using the *lrm* function in the *Design* library. Plant growth–related parameters were analysed using an ANOVA. Prior to the analysis, all plant growth–related variables were transformed in order to reduce skewness and achieve homogeneity in variance. R/S ratio, SRL and DNA content were transformed with the logarithm, and the remaining variables with the square-root. For the ANOVAs, the best model was selected according to Akaike Information Criterion (AIC) comparisons. In the survival analysis and the proportional odds model, the best model was selected using likelihood ratio tests that are asymptotically chi-squared distributed. Significant differences in the ANOVAS were further investigated using Tukey's HSD *post hoc* test.

Results

Koch's postulates were fulfilled for *P. subalpina* and the two pathogens. On average, *P. subalpina* was successfully re-isolated from 65% of all seedlings at low and 70% at elevated temperature, *P. plurivora* from 63% and 39% and *E. undulatum* from 90% and 77%.

Seedling survival and disease intensity

Survival and disease intensity varied considerably among the two temperature treatments, *P. subalpina* isolates, and the presence or absence of the two pathogens (Fig. 1). Mortality of plants inoculated with *P. subalpina* ranged from 0% to 50% at low and 10% to 60% at elevated temperature, when no pathogen was inoculated. In the absence of *P. subalpina,* mortality of seedlings inoculated with *P. plurivora* was 30% at low and 60% at high temperature, whereas mortality of seedlings inoculated with *E. undulatum* was 90% and 80% at the respective temperature (Fig. 1). Significance of the parameters was the same in the survival analysis and the proportional odds model (Table 1): elevated temperature, inoculation with *P. subalpina* and/or the two pathogens significantly increased mortality and disease intensity of Norway spruce seedlings. Moreover, the significant interaction between *P. subalpina* and pathogens (I × P) indicates that some *P. subalpina* isolates suppressed *P. plurivora* and/or *E. undulatum* disease more effectively than others. For example, inoculation of seedlings with the isolate 6_2_7v reduced mortality caused by *P. plurivora*, whereas inoculation with isolates 6_70_4, 6_8_7v, 6_16_1 and

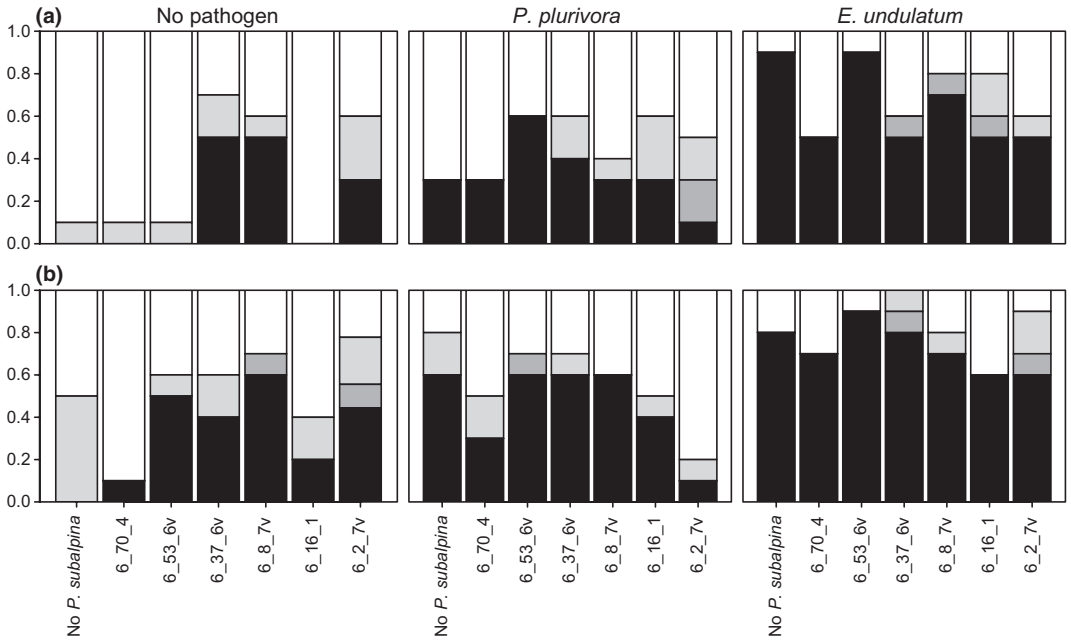

Fig. 1. Proportional distribution of the disease intensity of seedlings at (a) low and (b) elevated temperature inoculated with and without pathogens. *Phialocephala subalpina* isolate names and a *P. subalpina*-free control are indicated below each pathogen treatment (no pathogen, *Phytophthora plurivora* and *Elongisporangium undulatum*). Black indicates dead, dark grey heavily diseased, grey mildly diseased and white healthy seedlings. In total, there were $n = 10$ seedlings per treatment.

Table 1. Effects of isolate, temperature and presence of a pathogen on survival and disease intensity of the seedlings

		Survival analysis	Odds model
Parameter	df	Wald χ^2	Wald χ^2
Isolate	6	22.526**	46.004***
Temperature	1	8.114**	9.458**
Pathogen	2	93.114***	19.782***
Isolate × Pathogen	12	40.695***	28.771**

df, degrees of freedom.
**$P < 0.01$; ***$P < 0.001$.

6_2_7v reduced mortality caused by *E. undulatum* (Fig. 1). *Elongisporangium undulatum* was highly virulent on *P. subalpina*-free controls and seedlings inoculated with isolate 6_53_6v, resulting in survival of fewer than three seedlings at both temperatures (Figs 1 and 2). Elevated temperature led to a general increase in disease intensity, but this increase was the same for all *P. subalpina* isolates as indicated by the absence of a significant interaction between temperature and *P. subalpina*. Likewise, temperature did not alter the expression of disease symptoms caused by the two pathogens.

Plant growth–related parameters

Noninoculated seedlings (control) had the highest biomass (i.e. total biomass, needle, shoot, and root mass) at both temperatures. Biomass varied strongly between the two temperature regimes and among seedlings inoculated with different *P. subalpina* isolates and the two pathogens (Supporting Information, Table S1). Total biomass ranged from 4.96 to 368.9 mg, needle mass from 2.45 to 154.98 mg, shoot mass from 4.71 to 186.60 mg and root mass from 0.25 to 191.54 mg per plant. ANOVAs were performed for each pathogen separately. One analysis was performed with all seedlings inoculated with *P. plurivora* and any *P. subalpina* isolate (including the *P. subalpina*-free control inoculates) (analysis 1, Table 2a) and another one with a reduced data set containing the data of seedlings inoculated with *E. undulatum* comprising only those *P. subalpina* isolates (i.e. 6_16_1, 6_2_7v, 6_70_4, 6_8_7v) that led to survival of at least three seedlings at either temperature (analysis 2, Table 2b).

Analysis 1 showed that *P. plurivora* had no effect on biomass except for a slightly significant reduction in shoot weight, whereas elevated temperature and *P. subalpina* isolate significantly decreased all biomass parameters (Table 2a). Tukey's HSD test revealed that seedlings inoculated with *P. subalpina* isolate 6_2_7v had significantly lower biomass than the controls or seedlings inoculated with any other isolate. Moreover, biomass of seedlings inoculated with isolate 6_16_1 differed significantly from the controls at lower temperature. Significant

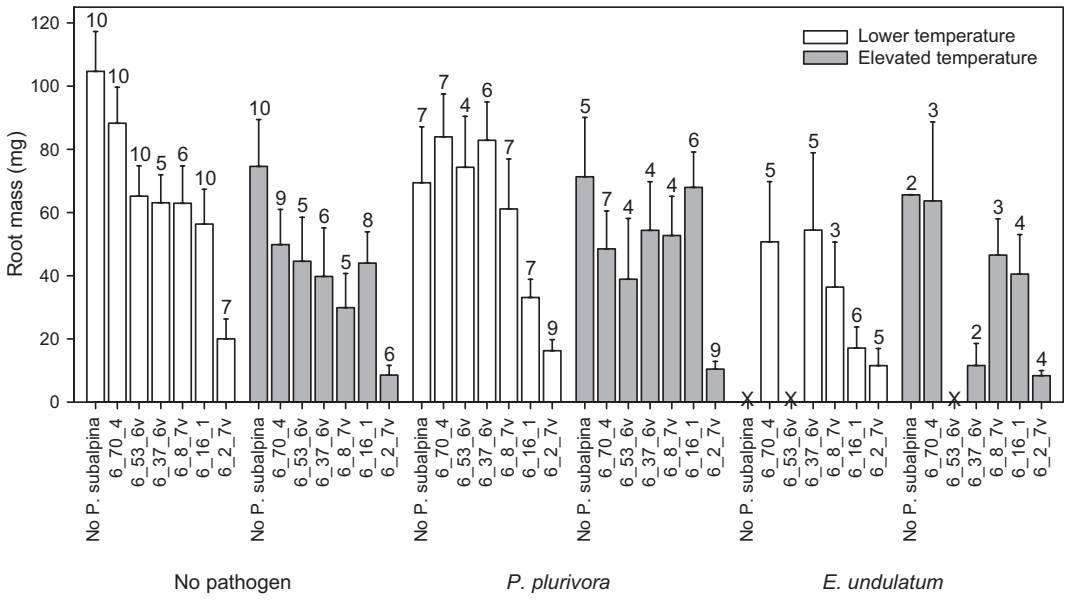

Fig. 2. Bar charts depicting the effect of temperature and inoculation with the root pathogens *Phytophthora plurivora* or *Elongisporangium undulatum* in combination with *Phialocephala subalpina* on mean root mass (± standard error) of Norway spruce seedlings. Root mass is grouped by pathogen treatment (no pathogen, *P. plurivora* and *E. undulatum*) and temperature regime. In total, there were $n = 10$ seedlings per treatment. Numbers above the bars indicate the number of surviving seedlings, and 'X' indicates treatments with less than two surviving seedlings.

Table 2. Effects of isolate, temperature and presence of a pathogen on plant growth–related parameters[‡]

	Total biomass	Needle mass	Shoot mass	Root mass	R/S ratio	SRL
	F	*F*	*F*	*F*	*F*	*F*
(a) Analysis 1: *P. plurivora*						
Temperature (T)	$33.226_{1,177}$***	$38.663_{1,183}$***	$42.009_{1,177}$***	$17.234_{1,183}$***	–	$21.897_{1,190}$***
P. subalpina (*sub*)	$21.895_{6,177}$***	$16.825_{6,183}$***	$20.212_{6,177}$***	$16.654_{6,183}$***	$6.511_{6,186}$***	–
P. plurivora (*pluri*)	$3.202_{1,177}$†	$3.392_{1,183}$†	$3.997_{1,177}$*	$1.815_{1,183}$	–	$2.032_{1,190}$
T × *sub*	$1.970_{6,177}$†	–	$2.042_{6,177}$	–	–	–
T × *pluri*	$3.289_{1,177}$†	$2.421_{1,183}$	$2.603_{1,177}$†	$3.123_{1,183}$†	–	–
(b) Analysis 2[§]: *E. undulatum*						
Temperature (T)	$9.168_{1,87}$**	$15.415_{1,87}$***	$15.028_{1,87}$***	$3.037_{1,87}$†	$1.412_{1,87}$	$8.836_{1,90}$**
P. subalpina (*sub*)	$19.448_{3,87}$***	$14.444_{3,87}$***	$16.793_{3,87}$***	$16.590_{3,87}$***	$7.037_{3,87}$***	–
E. undulatum (*und*)	$8.136_{1,87}$**	$5.188_{1,87}$*	$5.982_{1,87}$*	$8.707_{1,87}$**	$3.101_{1,87}$†	$0.640_{1,90}$
T × *und*	$10.014_{1,87}$**	$8.299_{1,87}$**	$8.266_{1,87}$**	$9.905_{1,87}$**	$2.351_{1,87}$	$4.855_{1,90}$*

Type III F–statistics from a linear model, followed by the effect and residual degrees of freedom (lowercase numbers).
†$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ – parameters excluded by model reduction according to the AIC criterion.
‡Interactions that were excluded by the AIC criterion for all plant growth–related parameters are not shown.
§Excluding *Phialocephala subalpina*-free seedlings and seedlings inoculated with *P. subalpina* isolates 6_37_6v and 6_53_6v (< 3 surviving seedlings).

differences among other isolates or the control were less consistent and depended on the biomass parameter.

In the reduced data set of analysis 2, inoculation with different *P. subalpina* isolates distinctly affected seedling biomass parameters. Likewise, all seedling biomass parameters were significantly decreased by inoculation with *E. undulatum*. In contrast, temperature significantly decreased total biomass, needle and shoot mass, but not root mass (Table 2b). However, the interaction between temperature and *E. undulatum* was significant for all four biomass parameters, indicating that temperature differently affected *E. undulatum*-free and infected seedlings at either temperature. *Post hoc* tests with Tukey's HSD revealed for each biomass parameter that this significant

interaction was due to a significant biomass reduction of *E. undulatum*-infected seedlings at low temperature, whereas there were no differences at elevated temperature between *E. undulatum*-free and infected seedlings. As in analysis 1, *P. subalpina* isolate 6_2_7v infected seedlings had significantly less biomass than the other seedlings in analysis 2. Moreover, there was a distinct protective effect of isolates 6_70_4, 6_8_7v, 6_16_1 and 6_2_7v against *E. undulatum* (Fig. 2). For any of these isolates, at least 30% of *E. undulatum*-infected seedlings survived, whereas almost all *P. subalpina*-free seedlings were killed by *E. undulatum* (Figs 1 and 2). Moreover, root mass of surviving *E. undulatum*-infected seedlings colonized by *P. subalpina* isolates 6_70_4 or 6_8_7v was almost as high or higher (at elevated temperature) as root mass of *E. undulatum*-free seedlings inoculated with the same *P. subalpina* isolate (Fig. 2).

The root-to-shoot (R/S) ratio ranged from 0.05 to 2.62 and differed significantly only among seedlings inoculated with different *P. subalpina* isolates in both ANOVAs (Table 2). This significant effect was due to a R/S ratio reduction by isolate 6_2_7v, indicating that seedlings infected with this particular isolate had proportionally less root than shoot mass compared to plants inoculated with the other isolates and the controls.

SRL ranged from 13.04 to 101.22 mm mg^{-1} and was significantly increased by elevated temperature for both pathogens (Table 2). However, there was a significant interaction between temperature and *E. undulatum*, which was due to differences in SRL at low and elevated temperature. These differences were significant for seedlings inoculated with *E. undulatum*, but not for *E. undulatum*-free seedlings.

Fungal colonization density

The amount of DNA of *P. subalpina* in the root samples was determined using a nested qPCR (Tellenbach *et al.*, 2010) and used as a measure for colonization density of *P. subalpina*. Colonization density varied among different *P. subalpina* isolates, but was within the same order of magnitude (Table 3). It was neither significantly affected by elevated temperature nor by the presence of a pathogen. After model reduction according to the AIC criterion, both *P. subalpina* isolate and pathogen were retained as factors, but only *P. subalpina* isolate was significant ($F_{5,100} = 8.99$, $P < 0.001$). Moreover, the absence of a significant pathogen by isolate interaction indicated that pathogen presence did not significantly alter endophyte colonization density. As revealed by Tukey's HSD test, isolate 6_2_7v had a significantly higher colonization density than all other isolates except isolate 6_8_7v (Table 3). Moreover, isolates 6_70_4 and 6_8_7v differed significantly from each other.

Discussion

Protection of Norway spruce by *P. subalpina* against oomycete root pathogens

The ability of six genetically distinct *P. subalpina* isolates to control two oomycete root pathogens of Norway spruce was tested *in vitro*. The degree of protection depended on *P. subalpina* isolate and pathogen, and the protective effect was not an artefact due to the failure of the pathogen to establish in the plants, because presence and viability of both pathogens were demonstrated by high re-isolation rates. To estimate the efficacy of *P. subalpina* to control the pathogens, disease was quantified by estimating incidence and severity and by measuring different plant growth parameters. All these components must be assessed to fully appreciate the ability of an endophyte to control disease. For instance, the number of surviving plants alone is a poor predictor for the fitness of a plant population as demonstrated for isolate 6_2_7v. This isolate conferred the best protection against the two pathogens in terms of mortality. However, biomass of the surviving seedlings was very poor. Under field conditions, these seedlings would certainly be outcompeted. In contrast, the nonaggressive isolate 6_70_4 also provided very good protection against both pathogens combined with good plant growth. Thus, there is no linear relationship

Table 3. Mean *Phialocephala subalpina* colonization density ± standard deviation (ng DNA per mg root dry weight) of each pathogen treatment at low and elevated temperature

	No pathogen		*P. plurivora*		*E. undulatum*	
Isolate	Low	Elevated	Low	Elevated	Low	Elevated
6_70_4	4.9 ± 2.7	3.7 ± 1.8	10.1 ± 7.5	10.2 ± 6	6.9 ± 2.3	6.9 ± 4.1
6_53_6v	7.7 ± 2.6	13.1 ± 6.7	11.9 ± 5.8	10.4 ± 7.2	7.8 ± 4.1	15.9 ± 5.4
6_37_6v	9.4 ± 2.1	9.2 ± 5.5	8.3 ± 9.8	6.9 ± 4.1	11.8 ± 3.5	15.1 ± 4.7
6_8_7v	17.4 ± 8.5	14.7 ± 4.5	15.6 ± 9.9	13.8 ± 6.2	19.7 ± 4.5	16.1 ± 5.8
6_16_1	15.2 ± 11.1	19.9 ± 10.1	21.0 ± 28.8	6.0 ± 4.2	22.7 ± 15.7	10.7 ± 5.9
6_2_7v	28.9 ± 16	17.9 ± 8.9	18.6 ± 13.6	40.3 ± 27.5	43.9 ± 9.8	25.5 ± 19.6

between interaction strength of *P. subalpina* and protection against root pathogens. This is also supported by the absence of a relationship between protective effects and *P. subalpina* colonization density, which was previously shown to be positively correlated with seedling mortality (Tellenbach *et al.*, 2011).

The six *P. subalpina* isolates used in this study were selected based on their virulence exhibited on Norway spruce seedlings in a previous experiment (Tellenbach *et al.*, 2011). The virulence of the selected isolates ranged from neutral to highly virulent, as this corresponds to a more natural situation where isolates differing in strength of interaction with their host co-occur within the same plot (Queloz *et al.*, 2011; Tellenbach *et al.*, 2011). Moreover, plant response to isolates differing in virulence is distinct (Niks & Marcel, 2009), which in turn might affect the interaction with pathogens. The effect of the *P. subalpina* isolates was similar in the current study compared to the study of Tellenbach *et al.* (2011) with some isolates being slightly more and others slightly less aggressive. However, comparability of these two studies might be limited because experimental conditions were not exactly the same (i.e. substrate composition, temperature regime, duration).

In general, the protection against pathogens by endophytes and nonpathogenic isolates of fungal pathogens occurs either directly by antagonism or indirectly by the induction of host resistance. Antagonism occurs when the fungus attacks and penetrates the hyphae of a pathogenic fungus (i.e. by mycoparasitism), when it sequesters antibiotics and/or toxins that limit pathogen growth or when it competes for nutrients or space in the host tissue. These different mechanisms of protection against pathogens have been well documented in agricultural systems (Whipps, 2001; Harman *et al.*, 2004; Tripathi *et al.*, 2008; Alabouvette *et al.*, 2009). In our experiment, we cannot determine the exact mechanisms of antagonism, but the data suggest that competition between *P. subalpina* and the two root pathogens for infection sites is not the sole protection mechanism as there was no relationship between *P. subalpina* colonization density and protective capability. Moreover, mechanical- and chemical-induced resistance is well known in conifers and could be another protection mechanism conferred to Norway spruce seedlings by *P. subalpina* (Bonello & Blodgett, 2003). But as there were no significant differences in SRL, indicating the formation of thicker cell walls (e.g. by thickening of the exodermis; Eissenstat & Achor, 1999), mechanical-induced resistance seems to be less likely. In contrast, the production of toxins with antifungal activities against *P. plurivora* and *E. undulatum* as shown for *Pinus strobus* needle endophytes in disc diffusion assays against *Saccharomyces cerevisiae* and *Microbotryum violaceum* (Sumarah *et al.*, 2011) is likely and needs further consideration.

In contrast to mycorrhizal fungi, PAC can colonize roots of all age classes and can thus also be detected in the bark of coarse roots close to the stem base of mature trees (Grünig *et al.*, 2008). PAC frequently colonizes root tips (Menkis *et al.*, 2004; Grünig *et al.*, 2008; Wagg *et al.*, 2008) where they have to compete with ectomycorrhizal fungi. Interestingly, the primary roots of fungus-free conifer seedlings planted into forest soil as bait are usually much faster colonized by PAC than by ectomycorrhizal fungi (Ahlich *et al.*, 1998). However, colonization of established ectomycorrhizae (ECM) by PAC is significantly less frequent than that of nonmycorrhizal root tips (Grünig *et al.*, 2008). Mycorrhizal fungi are known to protect plant seedlings against pathogens (Newsham *et al.*, 1995; Azcón-Aguilar & Barea, 1997; Whipps, 2004), and Richard *et al.* (1971) showed that ECM formed by *Suillus granulatus* prevented DSE (probably PAC) from colonizing and adversely affecting *Picea mariana* seedlings. Similarly, PAC colonization of ECM of *P. abies* and *Pseudotsuga menziesii* was less frequent and less dense than that of nonmycorrhizal roots (V. Reininger, unpublished). ECM certainly form a physical barrier against colonization by other fungi including PAC. However, as PAC seems to be the faster colonizers, they might play an important protective role against pathogens early in the life of a spruce seedling, but complete exploitation of the root might be controlled by delayed colonization by ECM.

The effect of elevated temperature

Control of either pathogen by any *P. subalpina* isolate was not influenced by elevated temperature. Moreover, the relative among-isolate differences in the reduction in plant growth remained the same at both temperatures, although plant biomass was reduced at higher temperature. Studies that examined the influence of temperature on tripartite interactions are scarce, and many of them dealt in fact with bipartite systems (Köhl *et al.*, 1999; Singh *et al.*, 2009; Thomson *et al.*, 2010). Nonetheless, some studies were carried out using true tripartite systems demonstrating dependency of plant protection from the interaction between temperature, other environmental parameters, pathogen isolate and antagonist species as shown for the protection of Douglas fir seedlings against *Fusarium oxysporum* or bean plants against *Botrytis cinerea* (Strobel & Sinclair, 1991; Hannusch & Boland, 1996).

Elevated temperature did not significantly affect *P. subalpina* colonization and its influence on Norway spruce. However, the effect of elevated temperature on endophytes and mycorrhiza seems to be ruled by a complex set of additional factors as contradictory effects were found in different studies (e.g. Staddon *et al.*, 2004; Ju

et al., 2006; Compant *et al.*, 2010; Antunes *et al.*, 2011; Brosi *et al.*, 2011), and whether temperature affects endophyte as well as mycorrhiza colonization and behaviour further depends on host species (Rillig *et al.*, 2002; Compant *et al.*, 2010; Olsrud *et al.*, 2010).

Neither *P. plurivora* nor *E. undulatum* disease intensity increased on Norway spruce at elevated temperature, although both pathogens show increased vegetative growth in culture at higher temperature. The growth optimum of *P. plurivora* lies at approximately 25 °C (Jung & Burgess, 2009), whereas the growth optimum of *E. undulatum* lies at 30 °C (Robertson, 1980). Sporangium production by *E. undulatum*, however, was shown to occur only at temperatures between 18 and 20 °C (Goldie-Smith, 1952), whilst nothing is known of the optimum temperature for sporangium production in *P. plurivora*. In oomycetes, the critical step for pathogenesis and rapid colonization of uninfected host tissues is the proliferation of asexual zoospores, which are considered the major infectious units (Erwin & Ribeiro, 1996). Therefore, reduced sporangium production might explain the better performance of surviving seedlings infected with *E. undulatum* at elevated temperature. Nonetheless, the mortality caused by *E. undulatum* at elevated temperature was still high. In contrast, elevated temperature did not aggravate *P. plurivora* disease symptoms of the seedlings, and it is therefore likely that *P. plurivora* does not pose a major threat to Norway spruce seedlings in temperate forests, when temperature increases.

In our experiment, Norway spruce seedling biomass was reduced by approximately 30% at elevated temperature, indicating that these seedlings were more stressed under this condition. Similarly, mean biomass of black spruce (*P. mariana*) seedlings grown at 30 °C/24 °C was reduced by about 60% compared to biomass of seedlings grown at 22 °C/16 °C (Way & Sage, 2008). Moreover, soil heating experiments showed bell-shaped growth curves for conifer trees with a maximum at around 20 °C soil temperature (Lopushinsky & Max, 1990). Therefore, seedlings outside this temperature range might be slightly stressed and develop some mechanisms to evade heat stress, which in turn might also induce disease resistance (Sandermann, 2004; Wang *et al.*, 2006).

Conclusions

In this study, we have demonstrated that *P. subalpina*, the most abundant and widespread PAC species, can reduce disease intensity caused by the two oomycete root rot pathogens *E. undulatum* and *P. plurivora* in Norway spruce seedlings. However, suppression of pathogens is strongly *P. subalpina* strain dependent, and only two of the six strains constantly reduced mortality (6_2_7v, which, however, had a strong negative impact on host biomass) or both mortality and adverse effects on plant growth (6_70_4). Therefore, protection provided by PAC against more harmful pathogens might prompt plants to tolerate PAC despite negative effects of some strains on plant growth and health, and could explain why this symbiosis is globally widely distributed (Ahlich & Sieber, 1996; Queloz *et al.*, 2011). Moreover, PAC might also play a stabilizing role in protecting Norway spruce against oomycete root pathogens under climate change. However, because this study was performed *in vitro*, the findings must be verified in field trials as well, and it is further not clear whether this protective effect also occurs in adult trees, which are densely colonized by PAC (Ahlich & Sieber, 1996; Sieber & Grünig, 2006; Grünig *et al.*, 2008). Our study also gives valuable insights into the interaction between plants and endophytes, because data on the effects of elevated temperature are still scarce and inconclusive (Compant *et al.*, 2010; Van der Putten *et al.*, 2010). However, there may be other confounding factors like host and endophyte genotype or species, or other biotic and abiotic factors that need further consideration, before any conclusions about the wide distribution of PAC and their role in natural ecosystems can be drawn. Consequently, the protection of plants against pathogens by fungal endophytes under changing environmental conditions is complex. In both cases, basic knowledge of the components leading to this complexity can only be gathered with experiments performed under strictly controlled conditions, and it will be challenging, setting up field experiments to study the PAC–host interaction in a more natural environment.

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References

Addy HD, Piercey MM & Currah RS (2005) Microfungal endophytes in roots. *Can J Bot* **83**: 1–13.

Ahlich K & Sieber TN (1996) The profusion of dark septate endophytic fungi in non-ectomycorrhizal fine roots of forest trees and shrubs. *New Phytol* **132**: 259–270.

Ahlich K, Rigling D, Holdenrieder O & Sieber TN (1998) Dark septate hyphomycetes in Swiss conifer forest soils surveyed using Norway-spruce seedlings as bait. *Soil Biol Biochem* **30**: 1069–1075.

Alabouvette C, Olivain C, Migheli Q & Steinberg C (2009) Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing *Fusarium oxysporum*. *New Phytol* **184**: 529–544.

Antunes PM, Koch AM, Morton JB, Rillig MC & Klironomos JN (2011) Evidence for functional divergence in arbuscular mycorrhizal fungi from contrasting climatic origins. *New Phytol* **189**: 507–514.

Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N & Herre EA (2003) Fungal endophytes limit pathogen damage in a tropical tree. *PNAS* **100**: 15649–15654.

Ayres MP & Lombardero MJ (2000) Assessing the consequences of global change for forest disturbance from herbivores and pathogens. *Sci Total Environ* **262**: 263–286.

Azcón-Aguilar C & Barea JM (1997) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. *Mycorrhiza* **6**: 457–464.

Bonello P & Blodgett JT (2003) *Pinus nigra-Sphaeropsis sapinea* as a model pathosystem to investigate local and systemic effects of fungal infection of pines. *Physiol Mol Plant Pathol* **63**: 249–261.

Brasier CM (1996) *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. *Ann For Sci* **53**: 347–358.

Brasier CM, Kirk SA, Delcan J, Cooke DEL, Jung T & Man in'tVeld WA (2004) *Phytophthora alni* sp nov and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. *Mycol Res* **108**: 1172–1184.

Brosi GB, McCulley RL, Bush LP, Nelson JA, Classen AT & Norby RJ (2011) Effects of multiple climate change factors on the tall fescue–fungal endophyte symbiosis: infection frequency and tissue chemistry. *New Phytol* **189**: 797–805.

Brundrett M (2004) Diversity and classification of mycorrhizal associations. *Biol Rev* **79**: 473–495.

Carroll G (1988) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology* **69**: 2–9.

Chavarriaga D, Bodles WJA, Leifert C, Belbahri L & Woodward S (2007) *Phytophthora cinnamomi* and other fine root pathogens in north temperate pine forests. *FEMS Microbiol Lett* **276**: 67–74.

Clay K (2001) Symbiosis and the regulation of communities. *Am Zool* **41**: 810–824.

Compant S, van der Heijden, MGA & Sessitsch A (2010) Climate change effects on beneficial plant-microorganism interactions. *FEMS Microbiol Ecol* **73**: 197–214.

Desprez-Loustau M-L, Robin C, Reynaud G, Déqué M, Badeau V, Piou D, Husson C & Marçais B (2007) Simulating the effects of a climate-change scenario on the geographical range and activity of forest-pathogenic fungi. *Can J Plant Pathol* **29**: 101–120.

Eissenstat DM & Achor DS (1999) Anatomical characteristics of roots of citrus rootstocks that vary in specific root length. *New Phytol* **141**: 309–321.

Erwin DC & Ribeiro OK (1996) *Phytophthora Diseases Worldwide*. APS Press, St. Paul., USA.

Garrett KA, Dendy SP, Frank EE, Rouse MN & Travers SE (2006) Climate change effects on plant disease: genomes to ecosystems. *Annu Rev Phytopathol* **44**: 489–509.

Goldie-Smith EK (1952) The sporangial phase of *Pythium undulatum* Petersen. *J Elisha Mitchell Sci Soc Chapel Hill N C* **68**: 273–292.

Grünig CR (2003) *Population Biology of the Tree-Root Endophyte Phialocephala fortinii*. Swiss Federal Institute of Technology, Zurich.

Grünig CR, Brunner PC, Duò A & Sieber TN (2007) Suitability of methods for species recognition in the *Phialocephala fortinii-Acephala applanata* species complex using DNA analysis. *Fungal Genet Biol* **44**: 773–788.

Grünig CR, Queloz V, Sieber TN & Holdenrieder O (2008) Dark septate endophytes (DSE) of the *Phialocephala fortinii* s.l. – *Acephala applanata* species complex in tree roots: classification, population biology, and ecology. *Botany* **86**: 1355–1369.

Hamm PB & Hansen EM (1982) Pathogenicity of *Phytophthora* species to Pacific Northwest conifers. *Eur J For Pathol* **12**: 167–174.

Hannusch DJ & Boland GJ (1996) Interactions of air temperature, relative humidity and biological control agents on grey mold of bean. *Eur J Plant Pathol* **102**: 133–142.

Harman GE, Howell CR, Viterbo A, Chet I & Lorito M (2004) *Trichoderma* species – opportunistic, avirulent plant symbionts. *Nat Rev Microbiol* **2**: 43–56.

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS & Samuel MD (2002) Ecology – climate warming and disease risks for terrestrial and marine biota. *Science* **296**: 2158–2162.

Hendrix FF & Campbell WA (1973) Pythiums as plant pathogens. *Annu Rev Phytopathol* **11**: 77–98.

Jeffers SN & Martin SB (1986) Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Dis* **70**: 1038–1043.

Johnson NC, Graham JH & Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytol* **135**: 575–585.

Ju HJ, Hill NS, Abbott T & Ingram KT (2006) Temperature influences on endophyte growth in tall fescue. *Crop Sci* **46**: 404–412.

Jumpponen A & Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytol* **140**: 295–310.

Jung T & Burgess TI (2009) Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and

North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia* **22**: 95–110.

Jung T, Blaschke H & Neumann P (1996) Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. *Eur J For Pathol* **26**: 253–272.

Jung T, Hudler GW, Jensen-Tracy SL, Griffiths HM, Fleischmann F & Osswald W (2005) Involvement of *Phytophthora* species in the decline of European beech in Europe and the USA. *Mycologist* **19**: 159–166.

Köhl J, Lombaers-van der Plas CH, Molhoek WML, Kessel GJT & Goossen-Van Der Geijn HM (1999) Competitive ability of the antagonists *Ulocladium atrum* and *Gliocladium roseum* at temperatures favourable for *Botrytis* spp. development. *Biocontrol* **44**: 329–346.

Lilja A (1994) The occurrence and pathogenicity of uni- and binucleate *Rhizoctonia* and *Pythiaceae* fungi among conifer seedlings in Finnish forest nurseries. *Eur J For Pathol* **24**: 181–192.

Lilja A, Lilja S, Poteri M & Ziren L (1992) Conifer seedling root fungi and root dieback in Finnish nurseries. *Scand J For Res* **7**: 547–556.

Lopushinsky W & Max TA (1990) Effect of soil temperature on root and shoot growth and on budburst timing in conifer seedling transplants. *New Forest* **4**: 107–124.

Menkis A, Allmer J, Vasiliauskas R, Lygis V, Stenlid J & Finlay R (2004) Ecology and molecular characterization of dark septate fungi from roots, living stems, coarse and fine woody debris. *Mycol Res* **108**: 965–973.

Miller J, Sumarah M & Adams G (2008) Effect of a rugulosin-producing endophyte in *Picea glauca* on *Choristoneura fumiferana*. *J Chem Ecol* **34**: 362–368.

Nechwatal J & Osswald W (2001) Comparative studies on the fine root status of healthy and declining spruce and beech trees in the Bavarian Alps and occurrence of *Phytophthora* and *Pythium* species. *Forest Pathol* **31**: 257–273.

Newhook FJ & Podger FD (1972) The role of *Phytophthora cinnamomi* in Australian and New Zealand forests. *Annu Rev Phytopathol* **10**: 299–326.

Newsham KK (2011) A meta-analysis of plant responses to dark septate root endophytes. *New Phytol* **190**: 783–793.

Newsham KK, Fitter AH & Watkinson AR (1995) Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends Ecol Evol* **10**: 407–411.

Niks RE & Marcel TC (2009) Nonhost and basal resistance: how to explain specificity? *New Phytol* **182**: 817–828.

Ohlemüller R, Gritti ES, Sykes MT & Thomas CD (2006) Quantifying components of risk for European woody species under climate change. *Glob Change Biol* **12**: 1788–1799.

Olsrud M, Carlsson BÅ, Svensson BM, Michelsen A & Melillo JM (2010) Responses of fungal root colonization, plant cover and leaf nutrients to long-term exposure to elevated atmospheric CO_2 and warming in a subarctic birch forest understory. *Glob Change Biol* **16**: 1820–1829.

Queloz V, Duò A, Sieber TN & Grünig CR (2010) Microsatellite size homoplasies and null alleles do not affect species diagnosis and population genetic analysis in a fungal species complex. *Mol Ecol Resour* **10**: 348–367.

Queloz V, Sieber TN, Holdenrieder O, McDonald BA & Grünig CR (2011) No biogeographical pattern for a root-associated fungal species complex. *Glob Ecol Biogeogr* **20**: 160–169.

R Development Core Team (2009) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Richard C, Fortin JA & Fortin A (1971) Protective effect of an ectomycorrhizal fungus against the root pathogen *Mycelium radicis atrovirens*. *Can J For Res* **1**: 246–251.

Rillig MC, Wright SF, Shaw MR & Field CB (2002) Artificial climate warming positively affects arbuscular mycorrhizae but decreases soil aggregate water stability in an annual grassland. *Oikos* **97**: 52–58.

Robertson GI (1980) The genus *Pythium* in New Zealand. *NZ J Bot* **18**: 73–102.

Rodriguez RJ, White JF Jr, Arnold AE & Redman RS (2009) Fungal endophytes: diversity and functional roles. *New Phytol* **182**: 314–330.

Saikkonen K, Faeth SH, Helander M & Sullivan TJ (1998) Fungal endophytes: a continuum of interactions with host plants. *Annu Rev Ecol Syst* **29**: 319–343.

Sandermann H (2004) Molecular ecotoxicology: from man-made pollutants to multiple environmental stresses. *Molecular Ecotoxicology of Plants*, Vol. 170. Springer, Berlin, pp. 1–16.

Selosse M-A, Baudoin E & Vandenkoornhuyse P (2004) Symbiotic microorganisms, a key for ecological success and protection of plants. *CR Biol* **327**: 639–648.

Shafizadeh S & Kavanagh JA (2005) Pathogenicity of *Phytophthora* species and *Pythium undulatum* isolated from *Abies procera* Christmas trees in Ireland. *Forest Pathol* **35**: 444–450.

Sieber TN (2002) Fungal root endophytes. *Plant Roots: The Hidden Half* (Waisel Y, Eshel A & Kafkafi U, eds), pp. 887–917. Marcel Dekker, New York and Basel.

Sieber TN (2007) Endophytic fungi in forest trees: are they mutualists? *Fungal Biol Rev* **21**: 75–89.

Sieber TN & Grünig CR (2006) Biodiversity of fungal root-endophyte communities and populations in particular of the dark septate endophyte *Phialocephala fortinii*. *Microbial Root Endophytes* (Schulz B, Boyle C & Sieber TN, eds), pp. 107–132. Springer, Berlin.

Singh DP, Backhouse D & Kristiansen P (2009) Interactions of temperature and water potential in displacement of *Fusarium pseudograminearum* from cereal residues by fungal antagonists. *Biol Control* **48**: 188–195.

Staddon PL, Gregersen R & Jakobsen I (2004) The response of two *Glomus* mycorrhizal fungi and a fine endophyte to elevated atmospheric CO_2, soil warming and drought. *Glob Change Biol* **10**: 1909–1921.

Stoyke G, Egger KN & Currah RS (1992) Characterization of sterile endophytic fungi from the mycorrhizae of subalpine plants. *Can J Bot* **70**: 2009–2016.

Strobel NE & Sinclair WA (1991) Influence of temperature and pathogen aggressiveness on biological control of *Fusarium* root-rot by *Laccaria bicolor* in Douglas-fir. *Phytopathology* **81**: 415–420.

Sumarah MW, Kesting JR, Sørensen D & Miller JD (2011) Antifungal metabolites from fungal endophytes of *Pinus strobus*. *Phytochemistry* **72**: 1833–1837.

Tellenbach C, Grünig CR & Sieber TN (2010) Suitability of quantitative real-time PCR to estimate biomass of fungal root endophytes. *Appl Environ Microbiol* **76**: 5764–5772.

Tellenbach C, Grünig CR & Sieber TN (2011) Negative effects on survival and performance of Norway spruce seedlings colonized by dark septate root endophytes are primarily isolate-dependent. *Environ Microbiol* **13**: 2508–2517.

Thomson LJ, Macfadyen S & Hoffmann AA (2010) Predicting the effects of climate change on natural enemies of agricultural pests. *Biol Control* **52**: 296–306.

Tripathi S, Kamal S, Sheramati I, Oelmuller R & Varma A (2008) Mycorrhizal fungi and other root endophytes as biocontrol agents against root pathogens. *Mycorrhiza* (Varma A, ed), pp. 281–306. Springer, Berlin, Heidelberg.

Uzuhashi S, Tojo M & Kakishima M (2010) Phylogeny of the genus *Pythium* and description of new genera. *Mycoscience* **51**: 337–365.

Van der Putten WH, Macel M & Visser ME (2010) Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. *Philos Trans R Soc Lond B Biol Sci* **365**: 2025–2034.

Wagg C, Pautler M, Massicotte HB & Peterson RL (2008) The co-occurrence of ectomycorrhizal, arbuscular mycorrhizal, and dark septate fungi in seedlings of four members of the Pinaceae. *Mycorrhiza* **18**: 103–110.

Walker JF, Aldrich-Wolfe L, Riffel A, Barbare H, Simpson NB, Trowbridge J & Jumpponen A (2011) Diverse Helotiales associated with the roots of three species of Arctic Ericaceae provide no evidence for host specificity. *New Phytol* **191**: 515–527.

Walther G-R (2010) Community and ecosystem responses to recent climate change. *Philos Trans R Soc Lond B Biol Sci* **365**: 2019–2024.

Wang CJK & Wilcox HE (1985) New species of ectendomycorrhizal and pseudomycorrhizal fungi - *Phialophora finlandia*, *Chloridium paucisporum*, and *Phialocephala fortinii*. *Mycologia* **77**: 951–958.

Wang D, Eyles A, Mandich D & Bonello P (2006) Systemic aspects of host-pathogen interactions in Austrian pine (*Pinus nigra)*: a proteomics approach. *Physiol Mol Plant Pathol* **68**: 149–157.

Way DA & Sage RF (2008) Elevated growth temperatures reduce the carbon gain of black spruce [*Picea mariana* (Mill.) B.S.P.]. *Glob Change Biol* **14**: 624–636.

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. *J Exp Bot* **52**: 487–511.

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Can J Bot* **82**: 1198–1227.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Mean values and standard deviations of different plant growth related parameters.

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